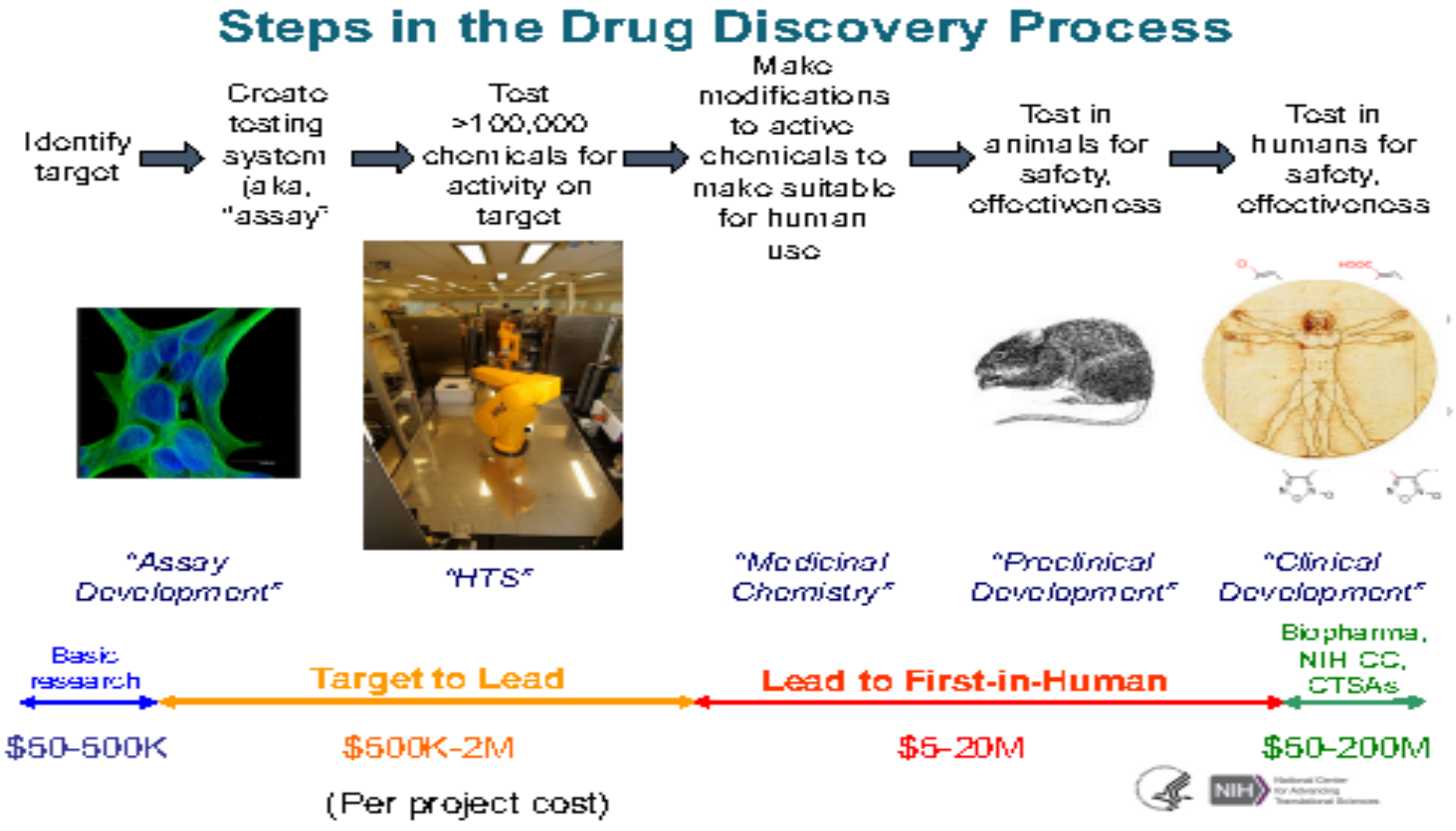
Early Therapeutic Discovery in Oncology and Beyond: Challenges and Opportunities

Anton Simeonov, Ph.D.

Scientific Director, National Center for Advancing Translational Sciences (NCATS), National Institutes of Health (NIH)

TRACO Lecture
September 28, 2020

Drug Discovery Process

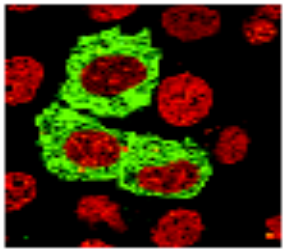


Range of screening assays

Range of Screening Assays

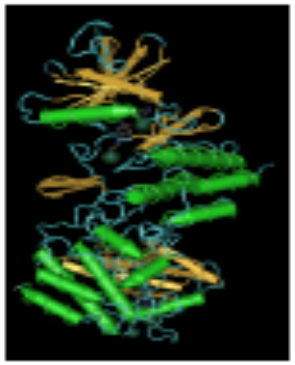
Extent of reductionism

Phenotype
(Image-based HCS, GFP, etc)

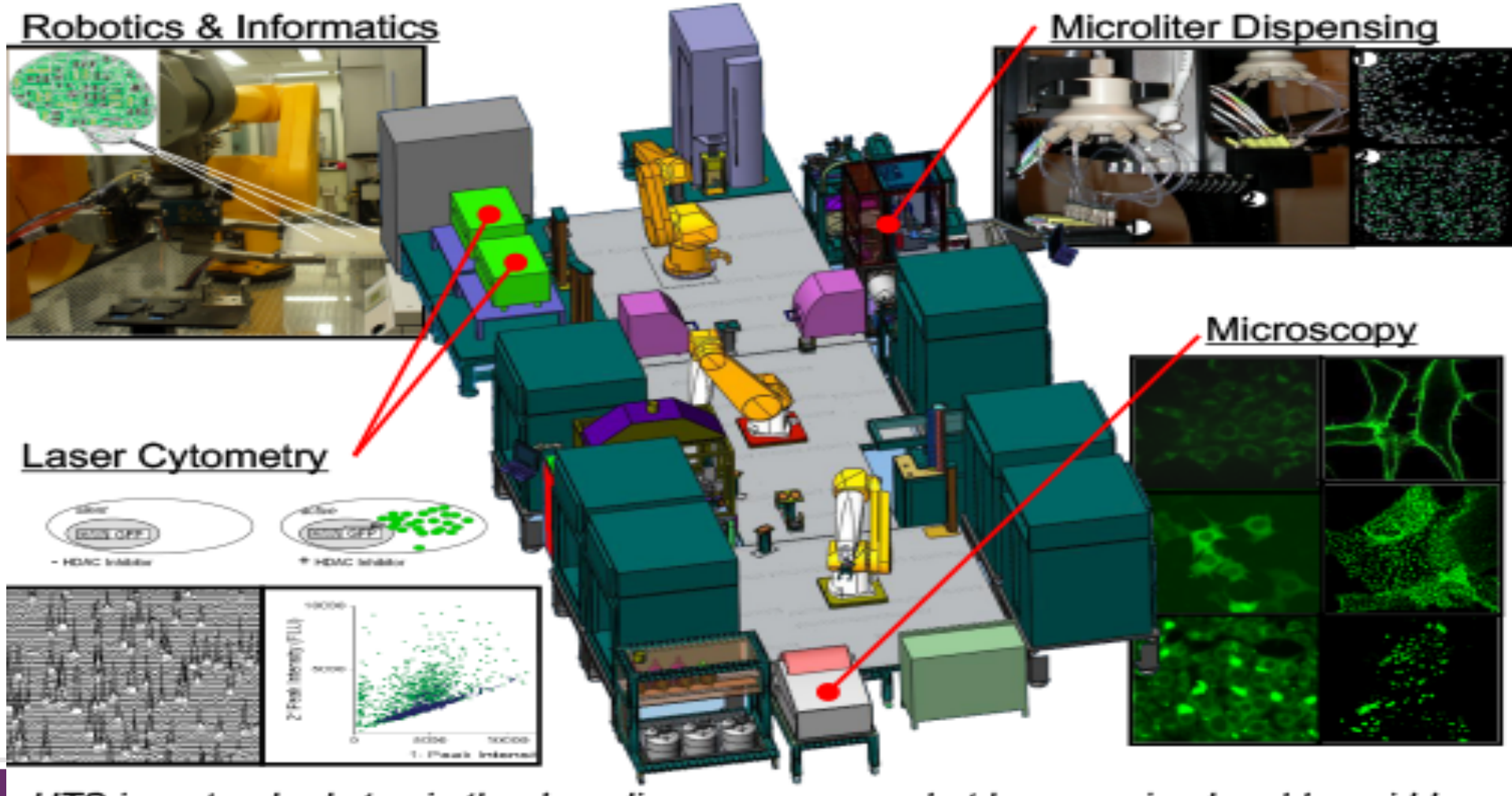


Pathway
(Reporters, e.g., luciferase, β-lactamase)

Protein
(Enzyme readouts, interactions, etc)

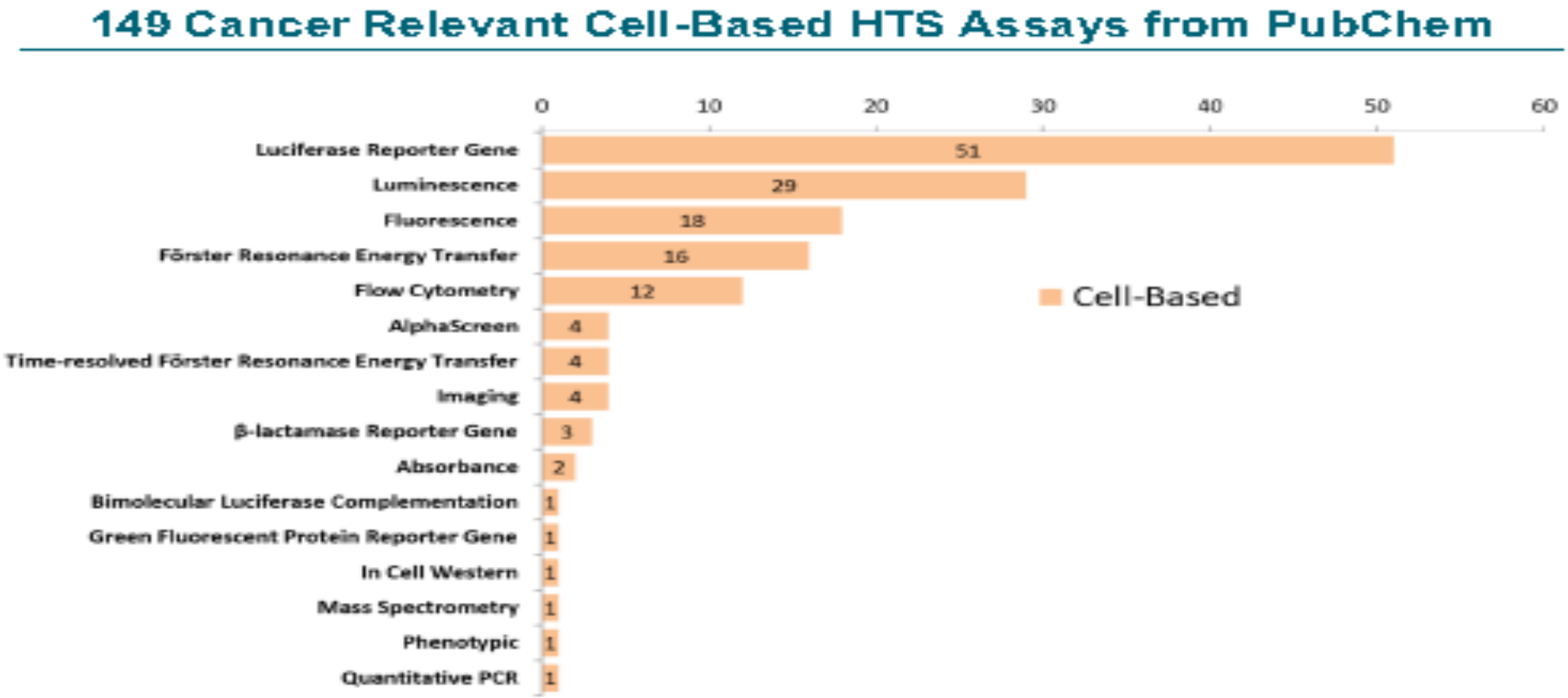


High throughput screening



HTS is a standard step in the drug discovery process but has remained problem-ridden.

Cell based HTS assays



Coussens, N. P., Braisted, J. C., Peryea, T., Sittampalam, S. G., Simeonov, A. and Hall, M. D. **Small Molecule Screens: A Gateway to Cancer Therapeutic Agents with Case Studies of FDA-Approved Drugs** *Pharmacological Reviews*, October 2017, 69 (4) 479-496

Assay choice

Important Considerations for Choosing an Assay

- Assay expense
  - Cost per well
  - Disposal cost(s)

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- **Available instrumentation**
  - Select the best possible assays based on the available instrumentation

Assay throughput

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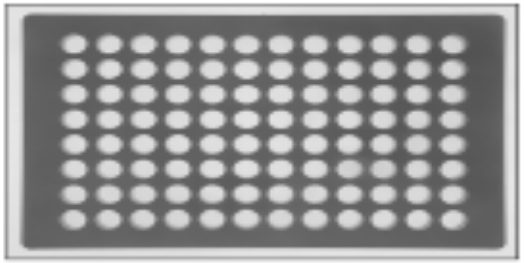
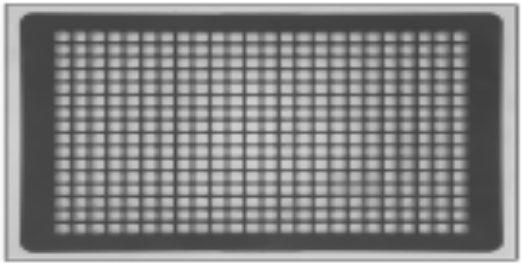
  - Select the best possible assays based on the available instrumentation
- **Assay throughput**
  - **Miniaturization reduces the cost per well**

Assay miniaturization

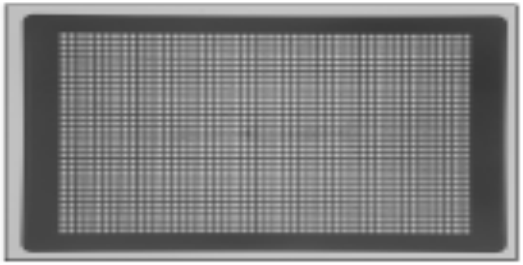
Assay Miniaturization Saves Time and Reagents



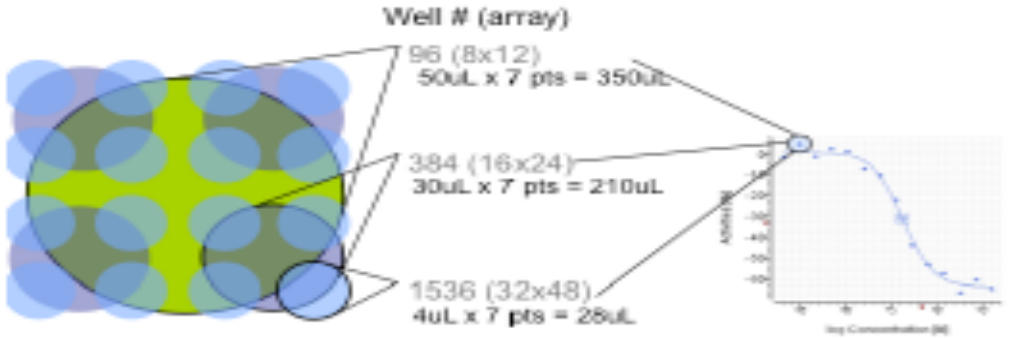
96-well
~50,000 cells/well



384-well
~5,000 cells/well



1536-well
~500 cells/well



	96	384	1536
Plates per 100,000 compounds:	1,042	261	66
Assay volume (uL):	50-200	30-50	2-8
Adherent cell seeding density:	~10,000	~2,000	~500

Iarman, Shane R. "Cancer High Content Phenotypic Screening." Cancer Targeted Drug Discovery. InTech, 2016.

Important considerations

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- Assay throughput
  - Miniaturization reduces the cost per well
- **Ability to multiplex**
  - Can the response be measured by a single parameter; is multiparametric output possible?
  - Increased data per sample
  - Can guide hit selection by differentiating selectivity among related targets
  - Can distinguish pathway inhibition from cytotoxicity in a cell-based assay

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- **Reagents**
  - **Stability for hours is important**
  - **Consistency is critical (ideally obtain a large quantity from a single lot)**
  - **All reagents need to be validated (cell lines, antibodies, enzymatic purity, etc.)**

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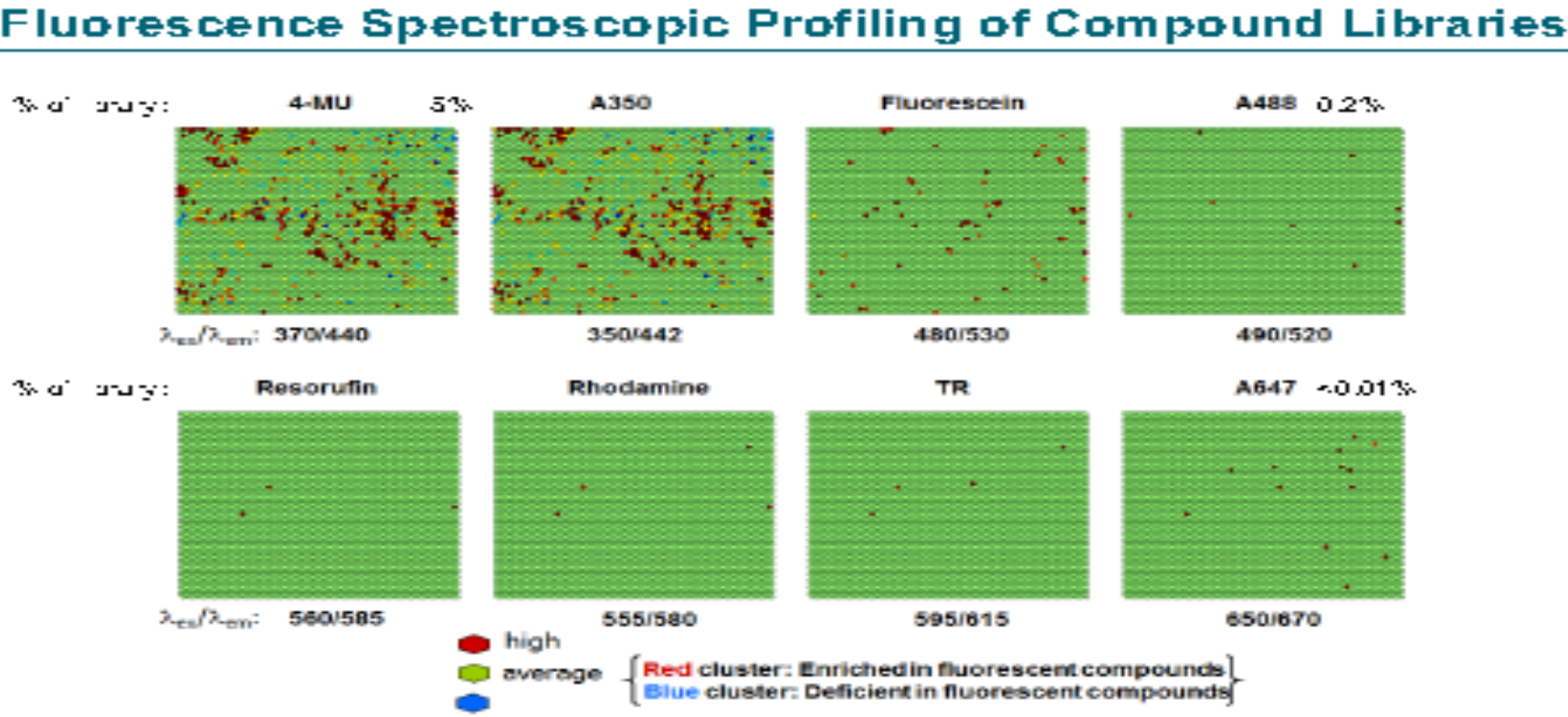
- Potential for assay interference
  - Fluorescent compounds can interfere with fluorescent readouts
  - Colored compounds might interfere with luminescence

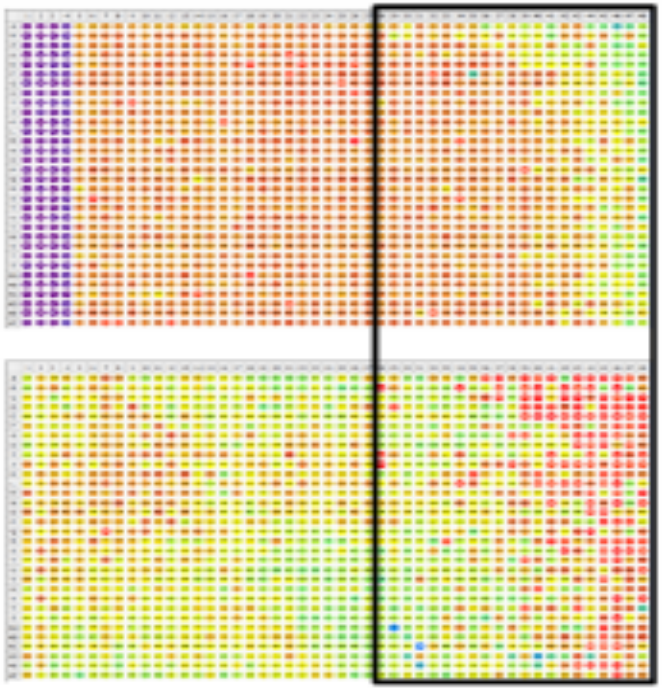
Fluorescence spectroscopic profiling

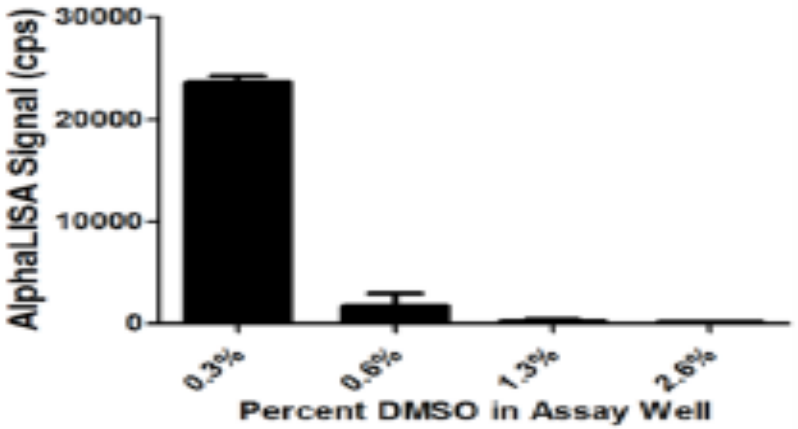


Simeonov, A., Jadhav, A., Thomas, C.J., Wang, Y., Huang, R., Southall, N.T., Shinn, P., Smith, J., Austin, C.P., Auld, D.S. and Inglese, J., 2008. **Fluorescence spectroscopic profiling of compound libraries.** *Journal of Medicinal Chemistry*, 51(8), 2363-2371.

Assay tolerance

Determination of Assay Tolerance to DMSO/Vehicle is Important





Yasgar A., Jadhav A., Simeonov A., Coussens N.P., AlphaScreen-Based Assays: Ultra-High-Throughput Screening for Small-Molecule Inhibitors of Challenging Enzymes and Protein-Protein Interactions. *Methods Mol Biol.* 2016;1439:77-98.

Important considerations

Important Considerations for Choosing an Assay

- Homogenous assay format is preferred for screening
  - Add reagents, mix and measure (no solution removal or wash steps)
  - Automation friendly
  - Reduces variability
  - Decreases hands-on time
  - Improves reproducibility

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- **Time required for assay**
  - Off-line reagent preparation
  - Is temperature equilibration required
  - Actual assay time
  - Kinetic versus end point read
  - Time required for data analysis and record keeping

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  - Longer signal stability allows for flexibility in automated systems
  - Longer signal stability minimizes differences among plates within a stack

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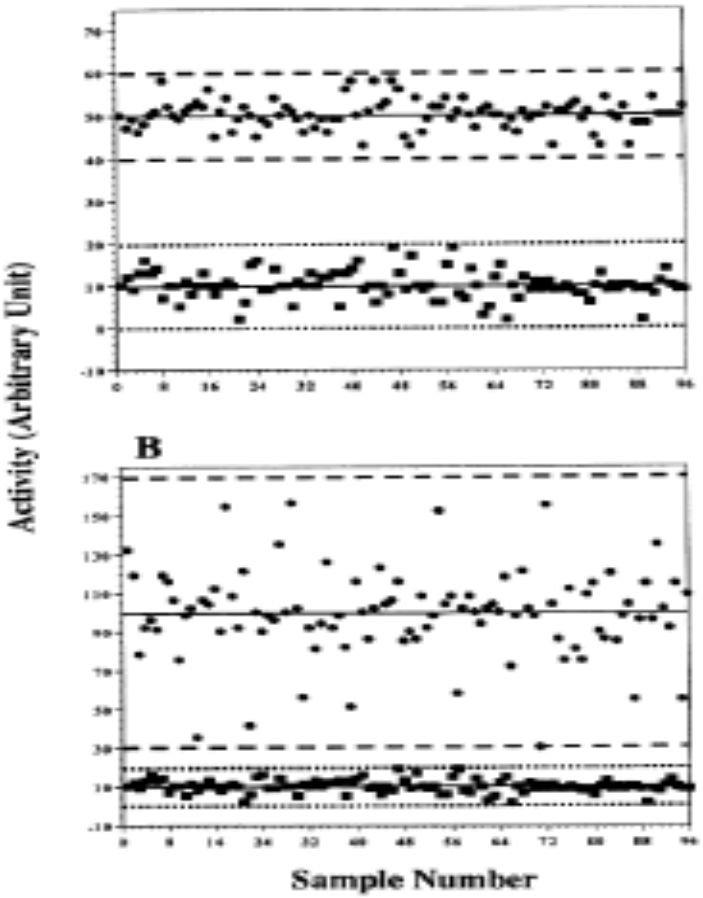
  - Longer signal stability minimizes differences among stacks of plates
- **Assay Sensitivity**
  - Choice of readouts is important
    - Colorimetric<fluorescent<luminescent

Assay suitability

Evaluating Assay Suitability for Screening



S/N = 12

S/B = 5

z = 0.5

S/N = 27

S/B = 10

z = 0.1

$$S/N = \frac{\text{mean signal} - \text{mean background}}{\text{standard deviation of background}}$$

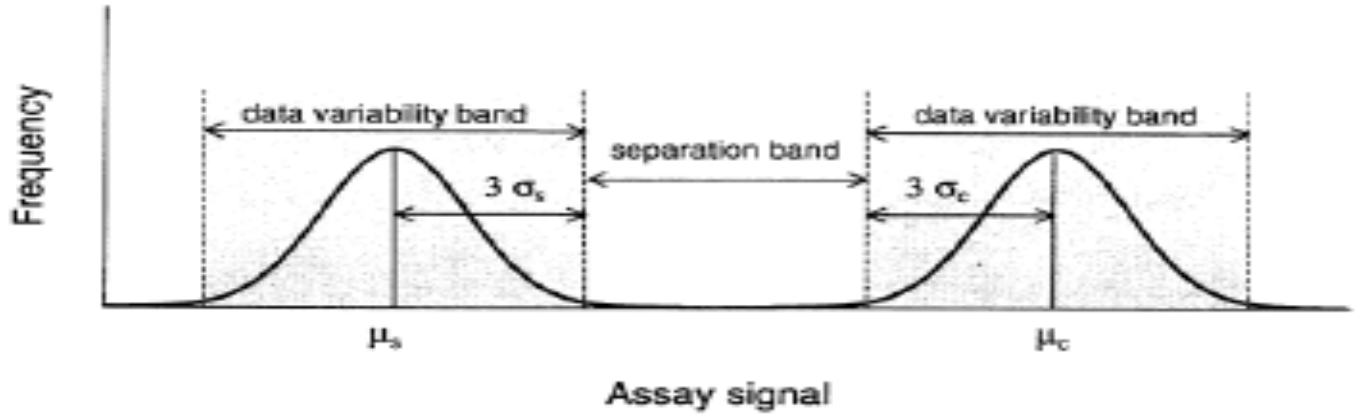
$$S/B = \frac{\text{mean signal}}{\text{mean background}}$$

$$Z = 1 - \frac{3SD \text{ of sample} + 3SD \text{ of control}}{|\text{mean of sample} - \text{mean of control}|}$$

A Simple Statistical Parameter for Use in Evaluation and Validation of High Throughput Screening Assays. Zhang JH, Chung TD, Oldenburg KR. J Biomol Screen. 1999;4(2):67-73.

Assay suitability

Evaluating Assay Suitability for Screening



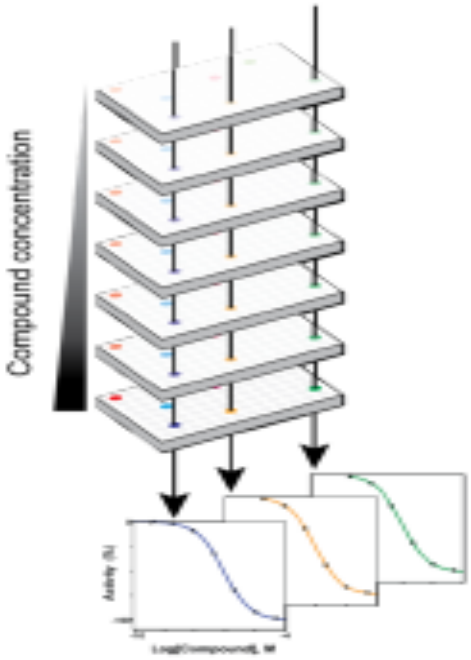
$$Z = 1 - \frac{3SD \text{ of sample} + 3SD \text{ of control}}{|\text{mean of sample} - \text{mean of control}|}$$

Z-factor value	*Structure of assay*	*Related to screening*
1	SD = 0 (no variation), or the dynamic range → ∞	An ideal assay
$1 > Z \geq 0.5$	Separation band is large	An excellent assay
$0.5 > Z > 0$	Separation band is small	A double assay
0	No separation band, the sample signal variation and control signal variation bands touch	A "yes/no" type assay
<0	No separation band, the sample signal variation and control signal variation bands overlap	Screening essentially impossible

A Simple Statistical Parameter for Use in Evaluation and Validation of High Throughput Screening Assays. Zhang JH, Chung TD, Oldenburg KR. J Biomol Screen. 1999;4(2):67-73.

Improving early discovery

Improving the Process of Early Discovery: Quantitative High-Throughput Screening (qHTS)



- Conventional screening done at one concentration
  - Not appropriate for potency testing – "dose makes the poison"
- qHTS tests compounds assayed at multiple concentrations (range: 4 logs)
- Enabled by miniaturized assay volumes (2-8 µL per test) and informatics pipeline
- Generates *pharmacological actives* instead of statistical "hits"
  - Dramatically increases reliability
  - Dramatically reduces false positives and false negatives
- *To date, several hundred million datapoints from several hundred screens have been generated and deposited in the public domain.*

PNAS 103:11473

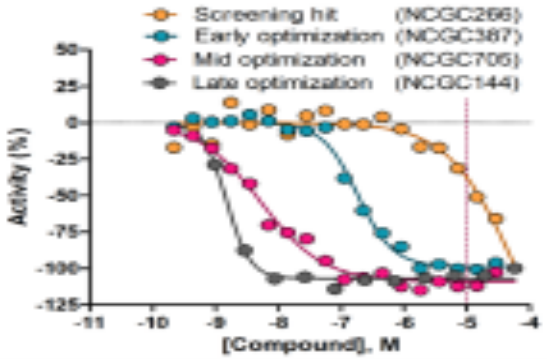
Medicinal chemistry

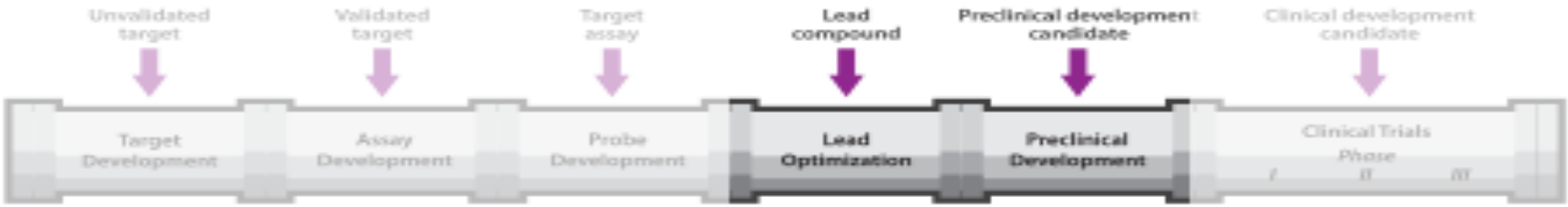
Medicinal Chemistry, an Integrated Process

Tier 1 : Synthesis & validation
- Medicinal chemistry
- Purification
- In vitro ADME

Tier 2: Compound profile expansion
- Met ID/ CYP studies
- In vitro toxicology

Tier 3: Advanced Preclinical studies
- Formulation
- Scale-up
- In vivo PK/PD
- Preclinical toxicology

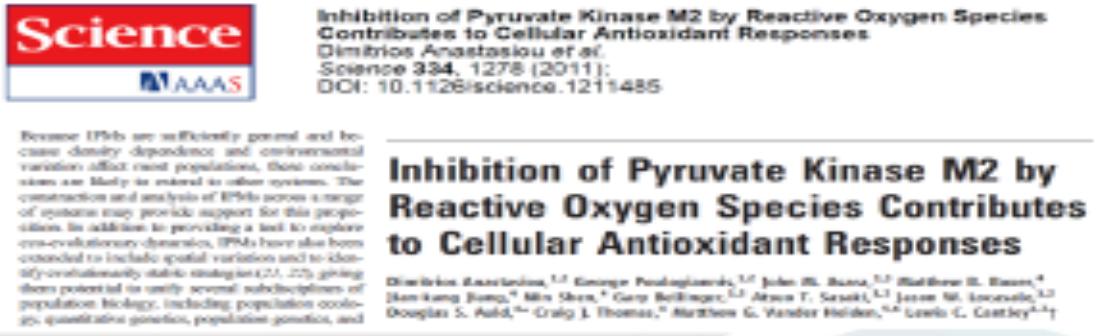




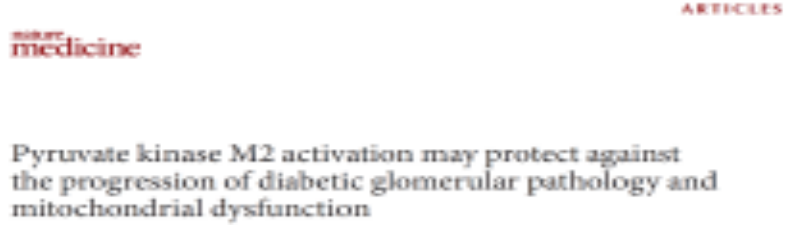
Small molecular probes

Example: small molecule probes to study cancer metabolism…with additional applications

- Tumors exhibit unique nutritional requirements. For example, pyruvate kinase M2 (PKM2) has been implicated in the Warburg Effect.
- A screen of PKM2, followed by medicinal chemistry optimization, produced several distinct molecules able to significantly activate the enzyme.
- These tool molecules have been used by numerous labs worldwide to study cancer biology and a subset are being further developed as therapies.

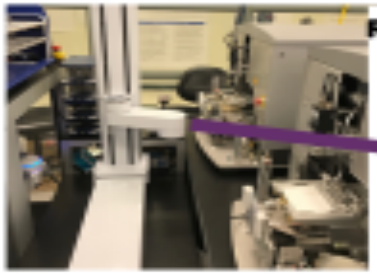


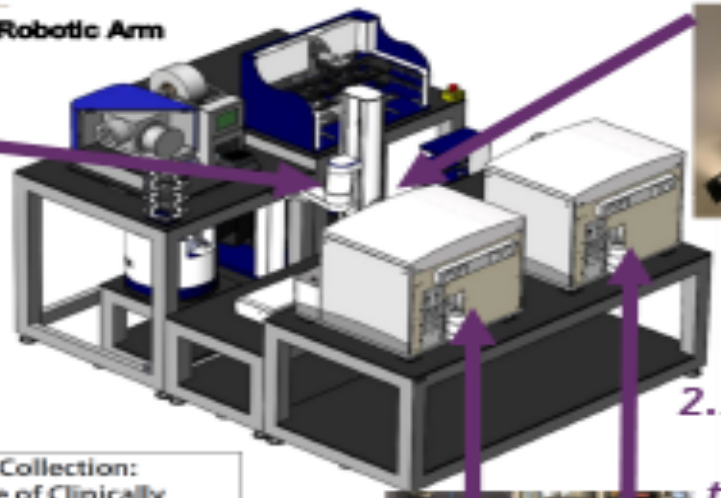
PKM2 activation may also be a way to modulate metabolism in pancreatic beta cells to manage diabetes:

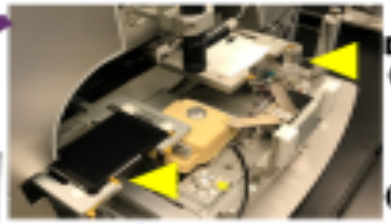


Drug combinations

Translation Challenge: Rapid Discovery of Drug Combinations







1. Appropriate libraries

2. Automation/ screening technologies

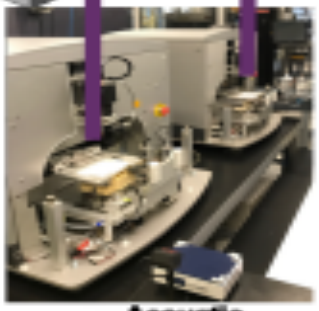
3. Informatics platform

The NCGC Pharmaceutical Collection: A Comprehensive Resource of Clinically Approved Drugs Enabling Repurposing and Chemical Genomics

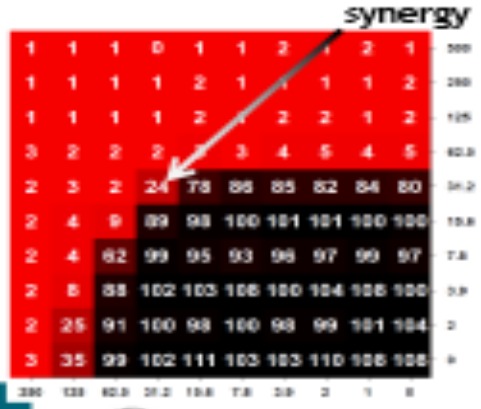
Ruili Huang, Noel Southall, Yuhong Wang, Adam Yasgar, Paul Shinn, Ajit Jadhav, Dac-Trung Nguyen, Christopher P. Austin

Small molecule compounds approved for use as drugs may be "repurposed" for new indications and studied to determine the mechanisms of their beneficial and adverse effects. A comprehensive collection of all small-molecule drugs approved for human use would be invaluable for systematic repurposing across human diseases, particularly for rare and neglected diseases, for which the cost and time required for development of a new chemical entity are often prohibitive. Previous efforts to build such a comprehensive collection have been limited by the complexities, redundancies, and semantic inconsistencies of drug naming within and among regulatory agencies worldwide; a lack of clear conceptualization of what constitutes a drug; and a lack of access to physical samples. We report here the creation of a definitive, complete, and non-redundant list of all approved molecular entities as a freely available electronic resource and a physical collection of small molecules accessible to high-throughput screening.

www.ScienceTranslationalMedicine.org 27 April 2011 Vol 3 Issue 80 80ps16

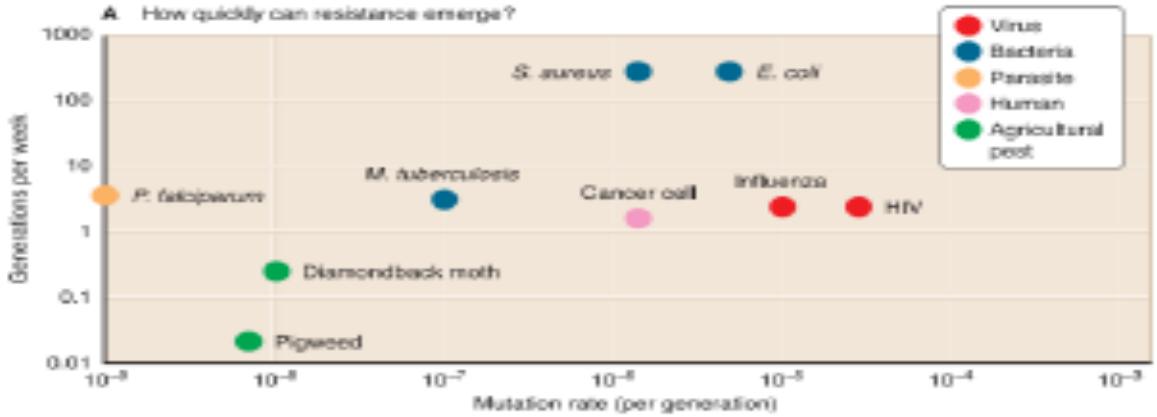


Acoustic Dispensers



Resistance

Application of Drug Combinations to Address Resistance



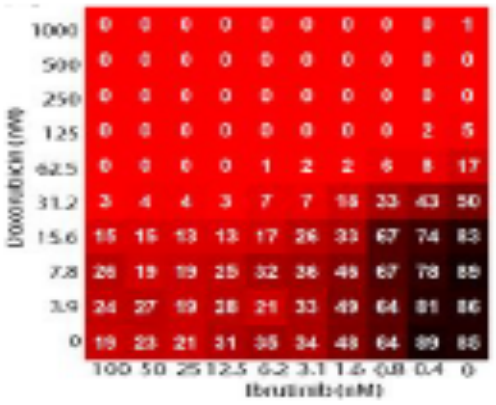
Drug resistance

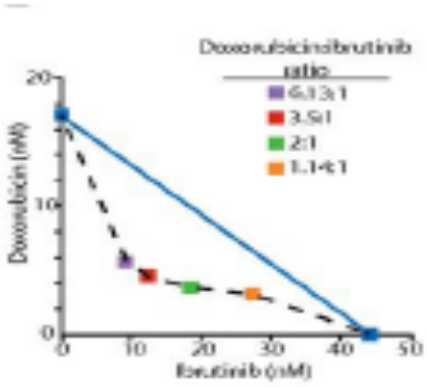
Dissemination of technology: combination screening to overcome drug resistance in cancer cells

- ABC subtype of Diffuse Large B-Cell Lymphoma (ABC-DLBCL) has poor prognosis and response to treatment
- Ibrutinib is a BTK inhibitor that has activity against ABC DLBCL
- Study evaluated 459 drugs in combination with Ibrutinib
  - 6 x 6 concentration-response "matrix blocks", validation in 10 x 10 concentration-response matrix blocks
- DNA-damaging agents identified as synergizing with Ibrutinib in killing ABC DLBCL cell lines
- **Dissemination:**
  - Protocols
  - Source code for dispense

High-throughput combinatorial screening identifies drugs that cooperate with ibrutinib to kill activated B-cell–like diffuse large B-cell lymphoma cells

Lesley A. Mathews Griner[a-1], Rajarshi Guha[a-1], Paul Shinn[a-1], Ryan M. Young[b-1], Jonathan M. Keller[a], Dongbo Liu[c], Ian S. Goldlust[a], Adam Yasgar[a], Crystal McKnight[a], Matthew B. Boxer[a], Damien Y. Duveau[a], Jian-Kang Jiang[a], Sam Michael[a], Tim Mierzwa[a], Wenwei Huang[a], Martin J. Walsh[a], Bryan T. Mott[a], Parseema Patel[a-1], William Leister[a], David J. Maloney[a], Christopher A. Lodish[a], Ganesha Rai[a], Ajit Jadhav[a], Brian D. Peyser[a], Christopher P. Austin[a], Scott E. Martin[a], Anton Simeonov[a], Marc Ferrer[a], Louis M. Staudt[b-1], and Craig J. Thomas[a-1]

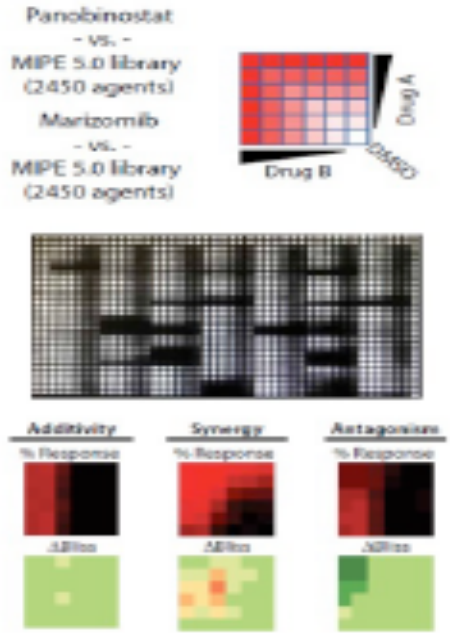




PNAS 111, 2349-2354

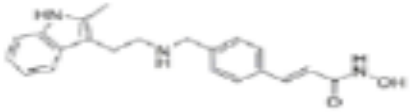
Rare brain tumors

Example: combination screening to tackle untreatable rare brain tumors

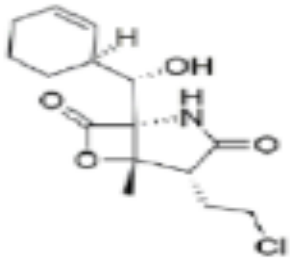


Lin *et al.*, *Sci. Transl. Med.* **11**, eaaw0064 (2019) 20 November 2019

Collaboration between NCATS (Craig Thomas) and Stanford University (Michelle Monje)



Panobinostat
HDAC inh.
Approved
MPO score: 4.82
Limited CNS pen.



Marizomib
Proteasome inh.
Phase 1
MPO score: 5.5
CNS penetrant

The team is currently exploring a clinical trial of panobinostat together with marizomib in DMGs.

SCIENCE TRANSLATIONAL MEDICINE | RESEARCH ARTICLE

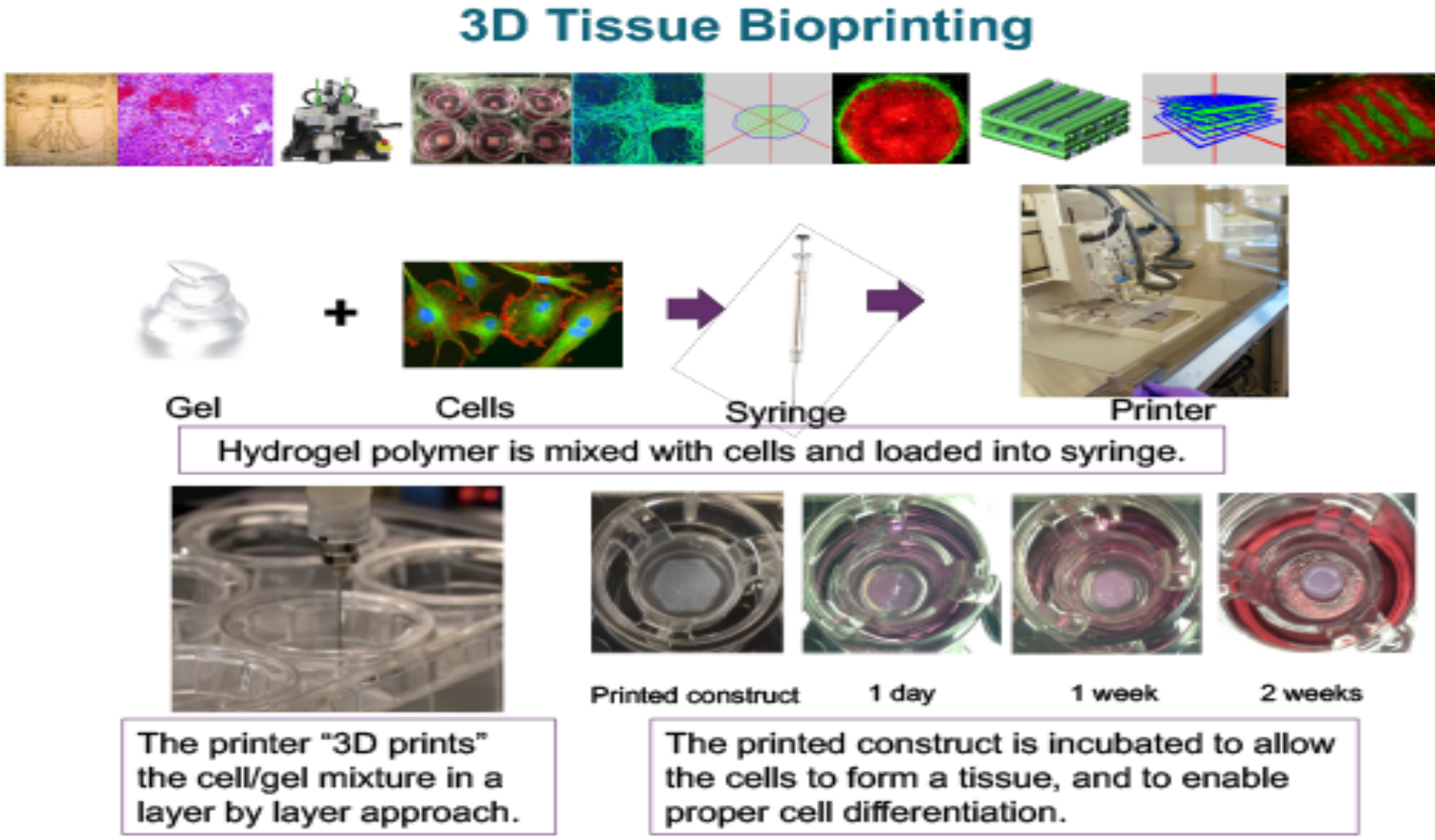
CANCER

Therapeutic strategies for diffuse midline glioma from high-throughput combination drug screening

3D models


Increasing the predictivity of *in vitro* assays: a continuum of 3D models of healthy and diseased tissues
2D
Spheroids
Organoids
Printed Tissues
Organ-on-a-chip
Physiological complexity
HTS compatibility

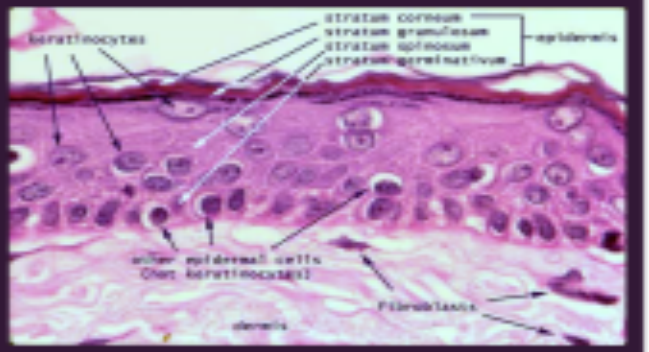
Tissue bioprinting



Epidermis Functional activity analyses. Stem cell technologies

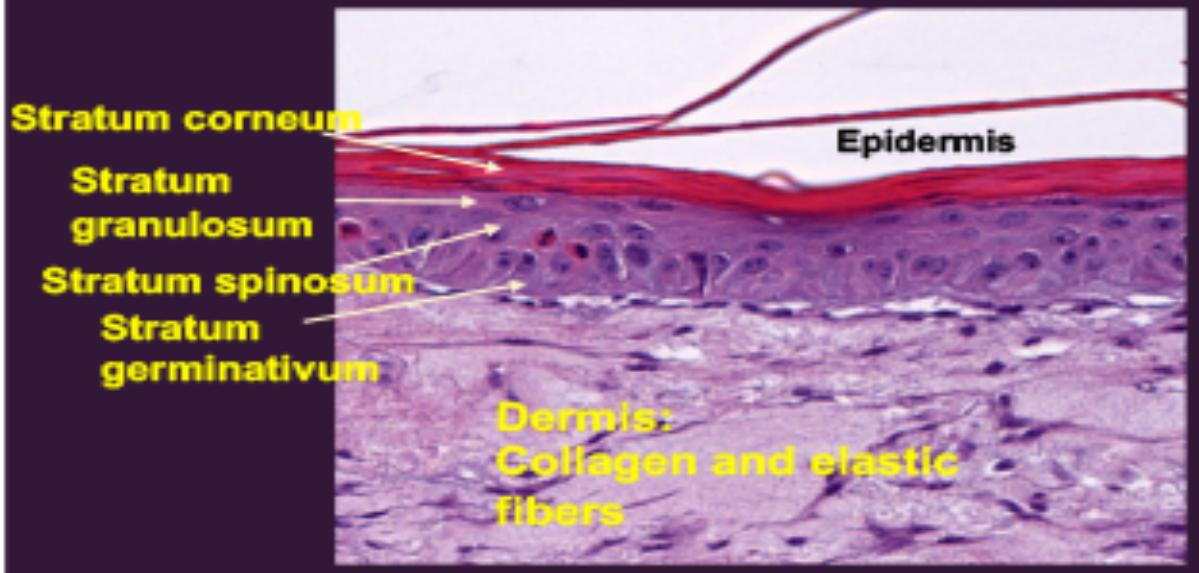
Layers of the Epidermis: native skin *versus* 3D-bioprinted skin

Native Skin



http://www.siumed.edu/~dking2/intro/IN005b.htm

3D-Bioprinted Skin

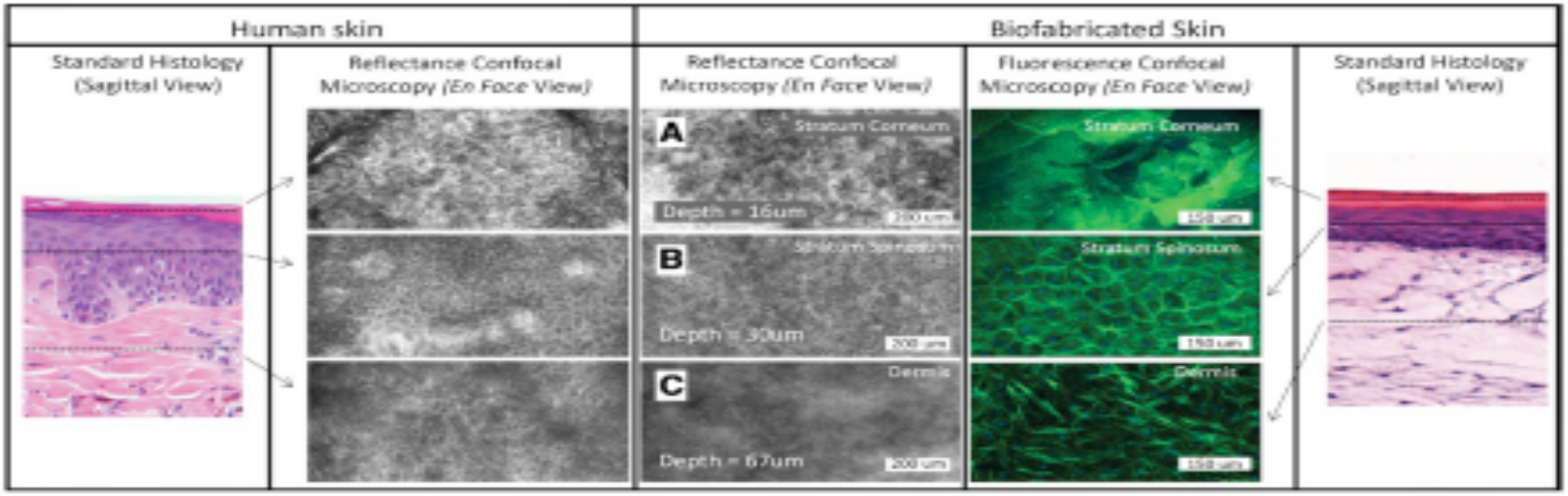


3D Tissue model

www.oncotarget.com Oncotarget, 2020, Vol. 11, (No. 27), pp: 2587-2596

Research Paper

A 3D biofabricated cutaneous squamous cell carcinoma tissue model with multi-channel confocal microscopy imaging biomarkers to quantify antitumor effects of chemotherapeutics in tissue

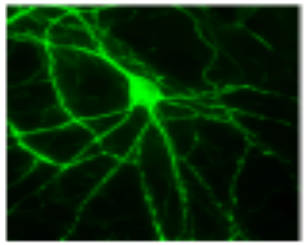


Collaboration between NCATS (Marc Ferrer) and Rockefeller University (Daniel Gareau)

Stem cell technologies

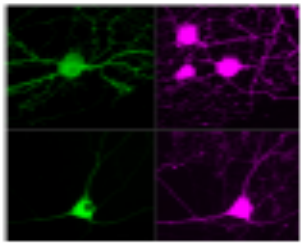
Enabling Advanced 3D Models and Regenerative Medicine through Stem Cell Technologies

NCATS Stem Cell Translation Laboratory:



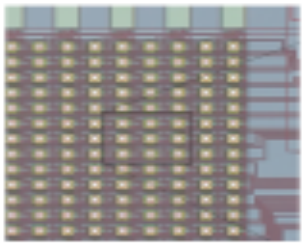
Access to relevant human cell types

Sensory neurons (nociceptors) and other neuronal subtypes, hepatocytes, etc.



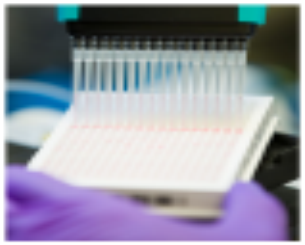
Advanced imaging technologies for functional cell characterization

High content confocal calcium imaging optogenetics



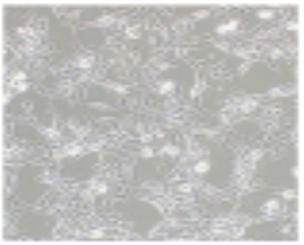
High-throughput electrophysiology methods

High density multi electrode arrays 24 x 96 electrode well



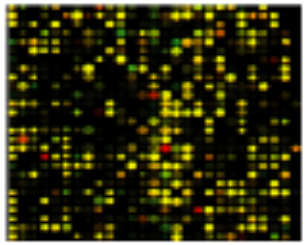
Measurement of signaling pathways, metabolism & specific targets

Cyclic AMP, PKA activity, CREB phosphorylation, energy metabolism



Longitudinal tracking of cell behavior

Multiple measurements over days, weeks or months



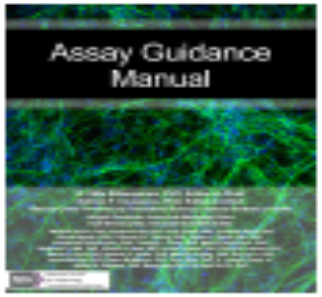
Combined single-cell transcriptomic & proteomic analyses

Drug response in individual cells

Assay development

Assay guidance manual

Sharing internal know-how: Assay Guidance Manual (47 chapters/ 1,338 printed pages)



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https://ncats.nih.gov/agm-video

August 2nd Video

1. Austin, CP: Welcome to the Assay Guidance Manual (AGM) Workshop
2. Comess, KM: Strategies for Assay Selection & Robust Biochemical Assays
3. Kahl, J: Freezing Cells as Reagents to Design Reproducible Screening Assays
4. Turek, CU: Assay Development Considerations for High Content Imaging
5. Auld, DS: Studies in Mechanisms and Methods in Assay Interferences
6. Dahlin, JL: Assay Interference by Chemical Reactivity
7. Chung, TDY: Basic Assay Statistics, Data Analysis & Rules of Thumb
8. Devanaryan, V: Reproducibility & Differentiability of Potency Results
9. Sittampalam, GS: Avoiding Artifacts & Interferences in Assay Operations

March 29-31st Video

1. Austin, CP: Welcome to the Assay Guidance Manual (AGM) Workshop
2. Comess, KM: Robust Assays Define Success in Preclinical Research
3. LaMarr, M: Target Identification & Validation in Translational Discovery
4. Foley, TL: Development & Validation of Cell-Based and Biochemical Assays
5. Kahl, J: Freezing Cells as Reagents to Design Reproducible Screening Assays
6. Turek, CU: Assay Development for HCS & Best Practices for 3D HCS
7. Roth, C: Mass Spectrometry for Drug Screening and Lead Optimization
8. Dahlin, JL: Bioassay Interference by Aggregation and Chemical Reactivity
9. Perry, S: Lead Selection and Optimization by Medicinal Chemistry
10. Xia, M: In Vitro Toxicological Testing Using qHTS Platform
11. Xu, X: In Vitro Assessment of ADME Properties of Lead Compounds
12. Carl, S: Statistical Design of Experiments for Assay Development
13. Guha, R: Pharos Application to Target Evaluation and Drug Discovery
14. Weidner, JR: Assay Operations: Keeping Assays Robust and Reproducible

Website: https://ncats.nih.gov/expertise/preclinical/agm

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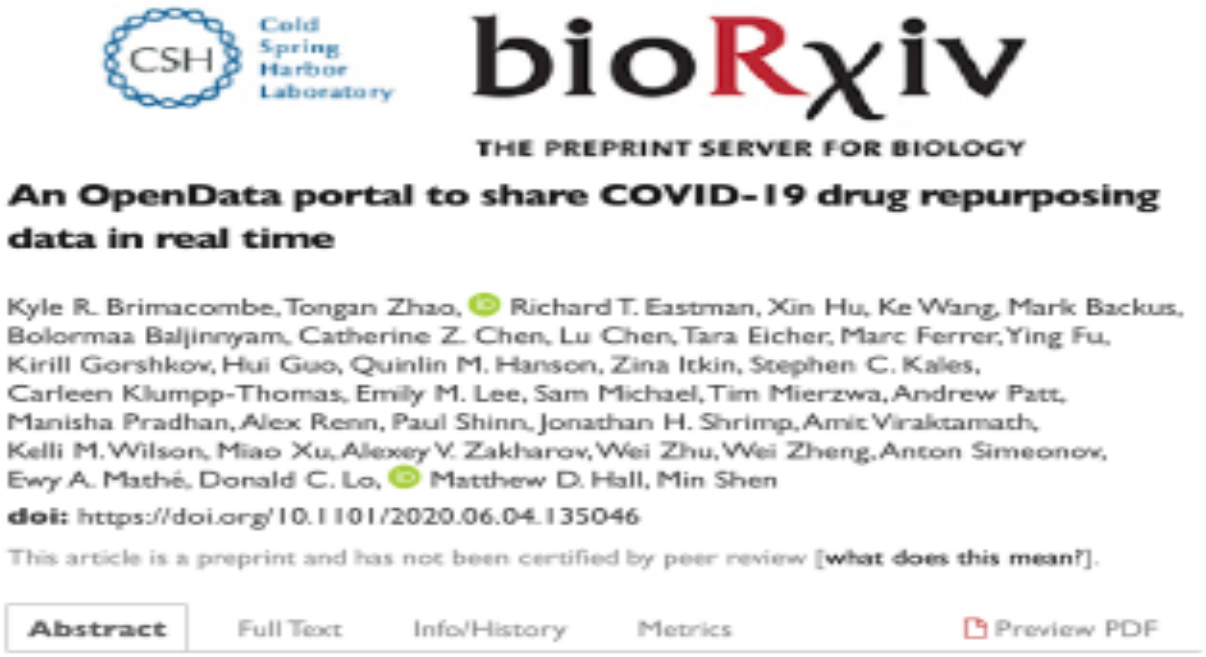
Response to CovID-19

Response to COVID-19: OpenData Portal enables data and protocol sharing in near-real time

https://opendata.ncats.nih.gov/covid19/

Open data portal



Cold Spring Harbor Laboratory

bioRxiv

THE PREPRINT SERVER FOR BIOLOGY

An OpenData portal to share COVID-19 drug repurposing data in real time

Kyle R. Brimacombe, Tongan Zhao, Richard T. Eastman, Xin Hu, Ke Wang, Mark Backus, Bolormaa Baljinnyam, Catherine Z. Chen, Lu Chen, Tara Eicher, Marc Ferrer, Ying Fu, Kirill Gorshkov, Hui Guo, Quinlin M. Hanson, Zina Itkin, Stephen C. Kales, Carleen Klumpp-Thomas, Emily M. Lee, Sam Michael, Tim Mierzwa, Andrew Patt, Manisha Pradhan, Alex Renn, Paul Shinn, Jonathan H. Shrimp, Amit Viraktamath, Kelli M. Wilson, Miao Xu, Alexey V. Zakharov, Wei Zhu, Wei Zheng, Anton Simeonov, Ewy A. Mathé, Donald C. Lo, Matthew D. Hall, Min Shen

doi: https://doi.org/10.1101/2020.06.04.135046

Abstract | Full Text | Info/History | Metrics | Preview PDF

Abstract

The National Center for Advancing Translational Sciences (NCATS) has developed an online open science data portal for its COVID-19 drug repurposing campaign – named OpenData – with the goal of making data across a range of SARS-CoV-2 related assays available in real-time. The

Multiple publicity, incl. featured on Francis Collins' blog post

NIH National Center for Advancing Translational Sciences

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National Center for Advancing Translational Sciences